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APPLICATION

FOR

UNITED STATES LETTERS PATENT

APPLICANT	:	GEORGE A. GAITANARIS
TITLE	:	VECTORS AND METHODS FOR THE MUTAGENESIS OF MAMMALIAN GENES

VECTORS AND METHODS FOR THE MUTAGENESIS OF MAMMALIAN GENES

Cross Reference to Related Applications

This application is a continuation of and claims priority from United States patent application 09/847,090, filed May 1, 2001 which is a divisional of U.S. utility application U.S.S.N. 09/002,046, filed December 31, 1997 (now U.S. Patent No. 6,228,639), which claims benefit from U.S. provisional application U.S.S.N. 60/034,094, filed December 31, 1996 (now abandoned).

Background of the Invention

This invention relates to retroviral vectors and their use in methods of mammalian gene mutagenesis.

Eukaryotic genomes are estimated to contain 6,000-80,000 genes (Collins, Proc. Natl. Acad. Sci. USA **92**:10821-10823 (1995)). Even in the best characterized organisms, the function of the majority of these genes is unknown. In addition, relatively little information is available concerning the fraction of the genome that is expressed in particular cell types or the cellular processes in which specific gene products participate. In an attempt to decipher genes' functions, large scale mutagenesis screens have been developed and have proven instrumental in unraveling the roles of certain genes in organisms such as *Drosophila melanogaster* (Nusslein-Volhard and Wieschaus, Nature **287**:795-801 (1980); Ballinger and Benzer, Proc. Natl. Acad. Sci. USA **86**:9402-9406 (1989); Kaiser and Goodwin, Proc. Natl. Acad. Sci. USA **87**:1686-1690 (1990); and Spradling et al., Proc. Natl. Acad. Sci. USA **92**:10824-10830 (1995)), *Caenorhabditis elegans* (Hirsh and Vanderslice, Dev Biol. **49**:220-235 (1976); and Zwaal et al., Proc. Natl. Acad. Sci. USA **90**:7431-7435 (1993)), *Zebrafish* (Solnica-Krezel et al., Genetics **136**:1401-1420 (1994); and Riley and Grunwald, Proc. Natl. Acad. Sci. USA **92**:5997-

6001 (1995)), *Arabidopsis* (Jurgens et al., Development Suppl. **1**:27-38 (1991); Mayer et al., Nature **353**:402-407 (1991); and Sundaresan et al., Genes Dev. **9**:1797-1810 (1995)), *Maize* (Scanlon et al., Genetics **136**:281-294 (1994); and Osborne and Baker, Curr. Opin. Cell Biol. **7**:406-413 (1995)), and *Saccharomyces cerevisiae* (Burns et al., Genes Dev. **8**: 1087-1105 (1994); and Chun and Goebl, Genetics **142**:30-50 (1996)). In mammals, however, these approaches have generally been limited by the large genome size and the development of the embryo inside a mother's uterus.

Some progress has been made in understanding mammalian gene function as a result of the development of mouse embryonic stem (ES) cell technology. This technology has significantly altered the field of mammalian genetics by allowing the bulk of genetic manipulations to be executed in vitro (Evans and Kaufman, Nature **292**:154-156 (1981); Bradley et al., Nature **309**:255-256 (1984); and Robertson, Trends Genet. **2**:9-13 (1986)). This is possible because mouse ES cells are pluripotent, that is, they have the ability to generate entirely ES cell-derived animals. Accordingly, gene inactivation in mouse ES cells and subsequent generation of "knock-out" (KO) mice is a powerful method for gaining information about the function of a gene in a whole animal system. If desired, genetic alterations, such as gene KOs which inactivate genes, may be introduced into these cells, and their consequences may be studied in the whole animal (Jaenisch, Science **240**:1468-1474 (1988); and Rossant and Nagy, Nat. Med. **1**:592-594 (1995)).

Currently, the available mouse mutagenesis methodologies are somewhat limited in their general utility as gene function screening systems. Gene targeting, the most widely used approach, is laborious and time consuming (Capecchi, Science **244**:1288-1292 (1989)). And gene trap and chemical/radiation induced mutagenesis are generally restricted in their targets (Gossler et al., Science **244**:463-465 (1989); Friedrich and Soriano, Genes Dev. **5**:1513-1523 (1991); Skarnes et al., Genes Dev. **6**:903-918 (1992); von Melchner et al., Genes Dev. **6**:919-927 (1992); Reddy et al., Proc. Natl. Acad. Sci.